201-15339B

IUCLID

Data Set

Existing Chemical	:	ID: 100-00-5
CAS No.	:	100-00-5
EINECS Name	:	1-chloro-4-nitrobenzene
EC No.	:	202-809-6
TSCA Name	:	Benzene, 1-chloro-4-nitro-
Molecular Formula	:	C6H4ClNO2

Producer related part

Company	:	Solutia
Creation date	:	24.04.2004

Substance related part

Company	:	Revised by: Toxicology and Regulatory Affairs Freeburg IL 62243 618-539-5280
Creation date	:	24.04.2004
Status	:	
Memo	:	p-CNB
Printing date	:	01.06.2004
Revision date	:	
Date of last update	:	01.06.2004
Number of pages	:	1
Chapter (profile)	:	Chapter: 1.0.1, 1.2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.1, 3.3.2, 3.5, 4.1, 4.2, 4.3, 4.4, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.7, 5.8.1, 5.8.2
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: SIDS

2. Physico-Chemical Data

Id 100-00-5
Date 01.06.2004

2.1 MELTING POINT

Value	:	= 83.4 °C
Sublimation	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	
Method	:	not referenced
Test substance	:	p-Nitrochlorobenzene (CASNO 100-00-5)
Reliability	:	(2) valid with restrictions Citation from a reputable, universally accepted reference guide; value cited in the PNCB HSDB (2002).
Flag	:	Critical study for SIDS endpoint

25.04.2004 (1)

2.2 BOILING POINT

Value	:	= 242 °C at 1013.25 hPa
Decomposition	:	
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	
Method	:	not reported
Remark	:	Listed as 242 deg. C @ 760 mm Hg.
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Citation from a reputable, universally accepted reference guide; value cited in the PNCB HSDB (2002).
Flag	:	Critical study for SIDS endpoint

31.05.2004 (1)

2.3 DENSITY

2.4 VAPOUR PRESSURE

Value	:	= .1253 hPa at 20 °C
Decomposition	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	
Remark	:	Reported as 0.094 mm Hg @ 20 deg. C.
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Cited as peer-reviewed in PNCB HSDB (2002).
Flag	:	Critical study for SIDS endpoint

31.05.2004 (2)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	= 2.39 at °C
pH value	:	
Method	:	other (measured)
Year	:	
GLP	:	no data
Test substance	:	
Method	:	Followed EPA methodology as defined in USEPA-600/4-79-032; Shake flask method using 6 replicates.
Test substance	:	p-Nitrochlorobenzene, CASNO 100-00-5
Reliability	:	(2) valid with restrictions Value derived from well accepted study design and consistent with other measured values reported in the literature (i.e. Hansch and Leo, 1995, SRC. Howard, 1990. Handbook of Environmental Fate and Exposure for Organic Chemicals. Lewis Pub.)
Flag	:	Critical study for SIDS endpoint

26.04.2004 (3)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= 189.4 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	
Method	:	Published value
Remark	:	Support for this value comes from a published estimate for the material's water solubility, where the "group contribution method" was used to estimate a water solubility of 154 mg/L (para) Kuehne, R, RU Ebert, F, Kleint, G. Schmidt and G. Schuurmann. 1995. Group contribution methods to estimate water solubility of organic chemicals. Chemosphere 30(11):2061-2077.
Result	:	An experimental value of 189.4 mg/L was reported in this publication.
Test substance	:	p-Nitrochlorobenzene (CASNO 100-00-5)
Reliability	:	(2) valid with restrictions Published value
Flag	:	Critical study for SIDS endpoint

25.04.2004 (4)

3.1.1 PHOTODEGRADATION

Type	:	other
Light source	:	Xenon lamp
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	
Conc. of sensitizer	:	
Rate constant	:	cm^3/(molecule*sec)
Degradation	:	98 % after 5 hour(s)
Deg. product	:	yes
Method	:	other (measured)
Year	:	1979
GLP	:	no data
Test substance	:	other TS

Method :

Photochemical reactivity assay where 1 mL of PNCB in n-hexane was put in 1 L reaction vessel, followed by substitution of n-hexane vapor with air or nitrogen free from nitrogen oxides. PNCB was deposited in the reaction vessel, which corresponded to 1000 ul gas if vaporized and was irradiated at 25-30 deg. C for 5 hr with the Xenon lamp (ozone-less type, Ushio co.).Disappearance of TS measured by HPLC. Reaction by-products measured by GC-MASS.

Result :

Rate of disappearance was influenced by the intensity of light passing through either of two reaction vessels used in this experiment, i.e. pyrex and quartz. The rate of disappearance of PNCB in air free of nitrogen, when tested in pyrex and quartz vessels, respectively, was 4.1% and 96%. When PNCB was tested in nitrogen free of nitrogen oxides in pyrex and quartz vessels, respectively, disappearance rates were 7.1% and 98%. The single reaction by-product identified in air free from nitrogen oxides was 4-Chloro-2-nitrophenol while p-chlorophenol was the only by-product identified in nitrogen free from nitrogen oxides.

Test substance :

p-Chloronitrobenzene (CASNO 100-00-5)

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
31.05.2004 (5)

Type	:	other
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm^3
Rate constant	:	.0000000000001714 cm^3/(molecule*sec)
Degradation	:	50 % after 62.4 day(s)
Deg. product	:	
Method	:	other (calculated)

Year	:	2002
GLP	:	no
Test substance	:	other TS
Method	:	Used AOPWIN, v. 1.90 from EPIWIN, Syracuse Research Corp.
Result	:	Vapor phase of PNCB is susceptible to reaction with photochemically-produced hydroxyl (OH) radicals. The 2nd order rate constant for reaction with hydroxyl radicals was calculated as 0.1714E-12 cm3(molecule*sec). Based on 1.5E6 OH molecules/cm3 and assuming 12 hours of sunlight per day, the estimated photo-oxidation half-life is 62.4 days (~1500 hrs).
Test substance	:	p-Nitrochlorobenzene
Reliability	:	(2) valid with restrictions Value obtained from EPA recommended estimation model.
Flag	:	Critical study for SIDS endpoint

31.05.2004 (6)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Degradation	:	< 50 % after 1 year at pH and °C
Method	:	Estimation on chemical principles

Hydrolysis of chloronitrobenzenes to chlorophenols (hydrolysis of the nitro group) or to nitrophenols (hydrolysis of the chloro group) are both thermodynamically feasible as the enthalpy of reaction calculated from bond energies indicates hydrolysis to be a thermodynamically favored process.

Hydrolysis of nitro group

Delta H =

300 kJ/mol (aromatic nitro group)
- 472 kJ/mol (phenol bond)

Total enthalpy = - 172 kJ/mol

Hydrolysis of chloro group

Delta H =

407 kJ/mol (aromatic nitro group)
- 472 kJ/mol (phenol bond)

Total enthalpy = - 65 kJ/mol

Although these reactions are thermodynamically favorable, the free energy of the transition state for this hydrolysis is so high that the reactions are generally not feasible (March's Advanced Organic Chemistry, fifth ed 2001 page 433).

It is known that special aromatic compounds, such as those with multiple electron withdrawing groups ortho and para to the halogen are potentially activated and can be substituted with difficulty. However, picryl chloride (with three nitro groups ortho or para to the chlorine) is stable in water at room temperature as it is transported with about 10% water to limit explosion potential. Thus, ortho and para CNB are anticipated to be hydrolysable under extreme conditions but are considered to have a hydrolytic half-life greater than one year under environmental conditions.

Bond energies from Lide, Handbook of Chemistry 84th edition 2003-2004 section 9

Result :

Considered to have a hydrolytic half-life greater than one year under environmental conditions.

Test substance :

p-Chloronitrobenzene [CAS No. 100-00-5]

Reliability : (2) valid with restrictions

Estimate by a reliable method

Flag : Critical study for SIDS endpoint

01.06.2004 (7)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media : other
Air : 9.52 % (Fugacity Model Level I)
Water : 28.5 % (Fugacity Model Level I)
Soil : 61.8 % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : .171 % (Fugacity Model Level II/III)
Method : other
Year :

Method :

Estimation using measured values from this dossier were incorporated into EPIWIN from Syracuse Research Corp., a methodology based on Meylan, 1993 as adopted by MacKay et al. 1996. Second Soil entry included estimation in Sediments. Values employed were : Mo. Wt = 157.56, vapor pressure of 0.094 mm Hg. Log Kow of 2.39, a melting point of 83 deg. C, and water solubility of 154 mg/L. Half lifes for air, water, soil and sediment were included as 1500 hr, 900 hr, 900 hr, and 3600 hr, respectively; emissions loading was 1000 kg/hr for each medium.

Remark :

Persistence Time was 506 hr.

Test substance :

p-Nitrochlorobenzene

Reliability : (2) valid with restrictions

Estimated values based on model recommended by US EPA.

Flag : Critical study for SIDS endpoint

31.05.2004 (6)

3.3.2 DISTRIBUTION

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	domestic sewage
Concentration	:	1 mg/l related to Test substance 10 mg/l related to Test substance
Contact time	:	
Degradation	:	34 - 66 (±) % after 24 hour(s)
Result	:	
Deg. product	:	
Method	:	other
Year	:	
GLP	:	no
Test substance	:	

Method :

Semi-Continuous Activated Sludge (SCAS) test conducted over 10-month period, in accordance with J Am Oil Chemists Society methods (JAOCS, 1965, 42:986 and JAOCS, 1965, 46:432). Inoculum was municipal waste treatment sludge. Feeding rate started at 1 mg/24-h and was raised in 1 mg increments to 5 mg over 28 days, and held at 5 mg/24-h for 4 months, then raised again to 10 mg/24-h. Twenty mL samples of mixed liquor (activated sludge + liquor) were taken 1 hr after each addition and at the end of the aeration cycle, via sidearm stopcock. The mixed liquor was extracted and analyzed via UV spectroscopy. Spike recovery experiments were 95.9 +/- 1.5%.

Result :

Average disappearance rate, days 75-120 (5 mg feed level, high aeration rate) was 33.9 +/- 2.9% over a 24-h cycle; over the next 60 days (same parameters) the disappearance rate was 30.7 +/- 9.4% over a 24-h cycle; over the last two weeks (10 mg feed level, low aeration), disappearance rate averaged 65.7 +/- 14.4% per 24-h cycle.

Test substance :

PNCB presumably as commercial grade with purity > 99%.

Reliability : (2) valid with restrictions

Study conducted prior to codification of GLPs but considered well documented. Methodology used has subsequently been incorporated into a standardized international test guideline for this study type.

Flag : Critical study for SIDS endpoint

25.04.2004 (8)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	= 1.8
LC50	:	= 6
Limit test	:	
Analytical monitoring	:	no
Method	:	other
Year	:	
GLP	:	yes
Test substance	:	other TS

Method :

Employed EPA methodology 660/3-75-009. Ten fish (ave. weight of 0.97 g and mean length of 40 mm), obtained from Trout Lodge, McMillin, WA, USA were tested in one of 5 test concentrations for up to 96-h. PNCB was administered in an acetone solution at concentrations of 1, 1.8, 3.2, 5.6 and 10 mg/L plus untreated and solvent control. Antimycin A was used as a positive control. Temperature was maintained at 12 +/- 1 deg. C. Tests were conducted in soft reconstituted deionized water, supplemented with 48 mg NaHCO3, 30 mg CaSO4, 30 mg MgSO4 and 2 mg KCL per liter. Fish were unfed 48 hr prior to testing and through the experimental period. Tests were conducted in 20-L glass vessels containing 15-L of solution. Dissolved oxygen was monitored to ensure the concentration did not fall below 2 mg/L before the end of the test. Water quality parameters such as pH, ammonia, and temperature were measured; no significant changes were observed during the test for these parameters. Estimation of LC50 and 95%CI were determined using EPA statistical procedures (probit analysis).

Remark :

Supporting ECOSAR Calculations are:

```
SMILES : c1c(CL)ccc(N(=O)(=O))c1
CHEM   : para-Chloronitrobenzene
CAS Num: 100-00-5
ChemID1:
ChemID2:
ChemID3:
MOL FOR: C6 H4 CL1 N1 O2
MOL WT : 157.56
Log Kow: 2.39  (User entered)
Melt Pt: 83.40 deg C
Wat Sol: 189 mg/L  (measured)

ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics


                                        Predicted
ECOSAR Class       Organism    Duration  End Pt mg/L (ppm)
```

```
================= ==========  ========  ====  ==========
Neutral Organic SAR : Fish        14-day  LC50  96.772
(Baseline Toxicity)

Neutral Organics   : Fish         96-hr   LC50  50.216
Neutral Organics   : Fish         14-day  LC50  96.772
Neutral Organics   : Daphnid      48-hr   LC50  55.276
Neutral Organics   : Green Algae  96-hr   EC50  35.342
Neutral Organics   : Fish         30-day  ChV    6.889
Neutral Organics   : Daphnid      16-day  EC50   3.362
Neutral Organics   : Green Algae  96-hr   ChV    4.428
Neutral Organics   : Fish  (SW)   96-hr   LC50  13.891
Neutral Organics   : Mysid Shrimp 96-hr   LC50  10.963
Neutral Organics   : Earthworm    14-day  LC50 735.052 *

 Note:  * = asterick designates: Chemical may not be soluble
        enough to measure this predicted effect.
         Fish and daphnid acute toxicity log Kow cutoff: 5.0
         Green algal EC50 toxicity log Kow cutoff: 6.4
         Chronic toxicity log Kow cutoff: 8.0
         MW cutoff: 1000
```

Calculated 2004, by Toxicology and Regulatory Affairs, Freeburg IL using measured Kow

Result :

The 96-h LC50 (95%CL) = 6.0 (4.8-7.6) mg/L.; the 48-h LC50 (95%CL) = 7.5 (6.1-9.2) mg/L; the 24-h LC50 (95% CL) = 8.8 (no CL calc.) mg/L. No deaths were observed up to 3.2 mg/L through 96 hrs. At the 5.6 mg/L level the following % mortality was reported at 24, 48 and 96-h: 0%, 10%, 50%. At 10 mg/L, mortality reached 70%, 90%, and 90% at 24, 48 and 96-h. Toxicity as exhibited by surfacing was seen at concentrations of 3.2 mg/L and higher beginning 24 hr after treatment while loss of equilibrium also was seen at 10 mg/L at all three time points. Dissolved oxygen ranged between 9.2-7.1 mg/L, pH between 7.2-7.6 and total nitrogen (NH3) of <0.1 - 0.3 mg/L.

Test substance :

PNCB (CASNO 100-00-5) with purity of > 99% (listed as 99.21%).

Reliability : (2) valid with restrictions

Well documented study which followed regulatory guidance for study conduct. LC50 value identical to that reported for guppies (Deneer et al. 1987. Aquat Toxicol 10:115) and similar to LC50 of 8.3 for bluegill sunfish (Solutia study no. AB-80-316)

Flag : Critical study for SIDS endpoint

31.05.2004 (9)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= 3.2
EC50	:	= 10
Analytical monitoring	:	no
Method	:	other
Year	:	
GLP	:	yes
Test substance	:	

Method :

Employed EPA methodology 660/3-75-009. Ten < 24-h old D. magna Straus (lab culture) were tested at 23 deg C in a series of three replicates per test concentration. PNCB in dimethyl formamide was tested at 6.25, 12.5, 25, 50 and 100 mg/L plus untreated control and solvent control. Morbidity and mortality were checked daily. Tests were conducted in 250-mL beakers containing 200 mL of solution. Well water from St Peter, MO, USA was used. Daphnids received no food 48-h prior to treatment. Water quality was measured to record dissolved oxygen, pH, alkalinity, hardness and temperature. Determination of EC50 and 95%CL were made using EPA statistical procedures (Steven, CE 1976. ASTM STP 634).

Remark :

Supporting ECOSAR Calculations are:

```
SMILES : c1c(CL)ccc(N(=O)(=O))c1
CHEM   : para-Chloronitrobenzene
CAS Num: 100-00-5
ChemID1:
ChemID2:
ChemID3:
MOL FOR: C6 H4 CL1 N1 O2
MOL WT : 157.56
Log Kow: 2.39  (User entered)
Melt Pt: 83.40 deg C
Wat Sol: 189 mg/L  (measured)

ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics
```

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organic SAR (Baseline Toxicity)	: Fish	14-day	LC50	96.772
Neutral Organics	: Fish	96-hr	LC50	50.216
Neutral Organics	: Fish	14-day	LC50	96.772
Neutral Organics	: Daphnid	48-hr	LC50	55.276
Neutral Organics	: Green Algae	96-hr	EC50	35.342
Neutral Organics	: Fish	30-day	ChV	6.889
Neutral Organics	: Daphnid	16-day	EC50	3.362

```
Neutral Organics   : Green Algae  96-hr   ChV    4.428
Neutral Organics   : Fish  (SW)   96-hr   LC50  13.891
Neutral Organics   : Mysid Shrimp 96-hr   LC50  10.963
Neutral Organics   : Earthworm    14-day  LC50 735.052 *

 Note:  * = asterick designates: Chemical may not be soluble
        enough to measure this predicted effect.
         Fish and daphnid acute toxicity log Kow cutoff: 5.0
         Green algal EC50 toxicity log Kow cutoff: 6.4
         Chronic toxicity log Kow cutoff: 8.0
         MW cutoff: 1000
```

Calculated 2004, by Toxicology and Regulatory Affairs, Freeburg IL using measured Kow

Result :

48-h EC50 (95% CL) = 11.1 mg/L (8.9-13.3); 24-h EC50 = 18.8 (16.9-21.1) mg/L. Dissolved oxygen ranged between 8.1-8.4 mg/L, pH was 7.0-8.1, alkalinity was 266-340 mg/L and hardness ranged between 226-318 mg/L. Temperature remained constant at 23 deg. C. The NOEC was < 6.25 mg/L. Per cent deaths seen at 24 and 48 hr respectively were : none in control or solvent control, 6.25 mg/L - 3%, 23%; 12.5 mg/L - 7%, 50%, 25 mg/L - 83%, 90%, 50 mg/L - 100% at both time points and at 100 mg/L - 100% deaths at both time points.

Test substance :

PNCB (CASNO 100-00-5) with purity of > 99%.

Reliability : (2) valid with restrictions

Well conducted study following regulatory accepted test guidelines. Solutia study (AB-80-317) using similar design and employing two replicates per dose resulted in 48-h EC50 of 10 (9-12) mg/L.

Flag : Critical study for SIDS endpoint

31.05.2004 (10)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	biomass
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC10	:	= 2.2
EC50	:	= 8
growth EC10	:	= 4.9
growth EC50	:	= 16
Method	:	other: DIN 38 412
Year	:	1988
GLP	:	no data
Test substance	:	

Method :

DIN 38412, Part 9 - The green alga S. subspicatus (Strain 8681 SAG) was used to conduct a modified cell multiplication inhibition test. A stock solution of the test substance was prepared in double-distilled water and diluted to prepare a series of test concentrations ranging from 0.80-100 mg/L.

The test was conducted in capped 250 ml Erlenmeyer flasks.
Eight (8) replicates of each concentration in defined algal growth media were tested.
Flasks were inoculated with the cell suspension (cell concentration of 10E5 cells/ml in each flask), placed on a white surface, protected from sunlight, shaken daily, and exposed to constant artificial lighting. The temperature was maintained at 24 +/- 1 deg C. and the relative humidity was 50%. A control group (8 replicates) was tested concurrently. On each measurement day, 50 ml were collected from each of two flasks from each test concentration or the control. The extinction value of the monochromatic radiation (578 nm wavelength) of the cell suspension was determined for each test concentration and the control.
Samples were collected and measurements were made at the beginning of the test and after 24 and 48 hrs. Biomass determination was based on measurement of optical density (turbidity). EC values were determined graphically by regression analysis.
The test used static conditions. Initial pH was adjusted to 8.0, final pH was measured but not reported.

Remark :

Supporting ECOSAR Calculations are:

```
SMILES : c1c(CL)ccc(N(=O)(=O))c1
CHEM   : para-Chloronitrobenzene
CAS Num: 100-00-5
ChemID1:
ChemID2:
ChemID3:
MOL FOR: C6 H4 CL1 N1 O2
MOL WT : 157.56
Log Kow: 2.39  (User entered)
Melt Pt: 83.40 deg C
Wat Sol: 189 mg/L  (measured)

ECOSAR v0.99f Class(es) Found
-----------------------------
Neutral Organics
```

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organic SAR (Baseline Toxicity)	Fish	14-day	LC50	96.772
Neutral Organics	Fish	96-hr	LC50	50.216
Neutral Organics	Fish	14-day	LC50	96.772
Neutral Organics	Daphnid	48-hr	LC50	55.276
Neutral Organics	Green Algae	96-hr	EC50	35.342
Neutral Organics	Fish	30-day	ChV	6.889
Neutral Organics	Daphnid	16-day	EC50	3.362
Neutral Organics	Green Algae	96-hr	ChV	4.428
Neutral Organics	Fish (SW)	96-hr	LC50	13.891
Neutral Organics	Mysid Shrimp	96-hr	LC50	10.963
Neutral Organics	Earthworm	14-day	LC50	735.052 *

```
 Note:  * = asterick designates: Chemical may not be soluble
        enough to measure this predicted effect.
         Fish and daphnid acute toxicity log Kow cutoff: 5.0
         Green algal EC50 toxicity log Kow cutoff: 6.4
         Chronic toxicity log Kow cutoff: 8.0
         MW cutoff: 1000
```

Calculated 2004, by Toxicology and Regulatory Affairs, Freeburg IL using measured Kow

Result :

Mean measured values of control group at 48 hrs were extinction value - 0.068; Biomass - 3.6 x 10E5 cells/ml. Results of the cell multiplication inhibition test of PNCB were: 48-h Biomass EC10 = 2.2 mg/L; 48-h Biomass EC50 = 8.0 mg/L. The 48-h average specific growth rate EC10 = 4.9 mg/l; 48-h average specific growth rate EC50 = 16 mg/L.

Test substance :

p-Nitrochlorobenzene (CASNO 100-00-5), purity unspecified.

Reliability : (2) valid with restrictions

Small deviations from standard study design, including shorter duration used (48 vs 72 h), and limited information presented on each test concentration at each measurement point.

Flag : Critical study for SIDS endpoint

31.05.2004 (11)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 530 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	20
Vehicle	:	other
Doses	:	
Method	:	other
Year	:	
GLP	:	no
Test substance	:	

Method :

Methodology similar to OECD # 401, except with fewer animals; PNCB was administered by gavage in 10% corn oil to groups of 5 mixed sex SD rats at dosages of 398, 501, 631 and 794 mg/kg. Animals were observed for signs of toxicity and death daily for 14 days. Body weights were recorded on study day 0 and weekly thereafter. Animals dying and all survivors to d14 were necropsied. Food and water were given ad libitum and temp., humidity and light were controlled. LD50 and CI were calculated by the method of deBeer, J. Pharmacol Experiment Ther 86:1.

Result :

LD50=530 mg/kg with CI of 480-590 mg/kg; Incidence of deaths observed at each dose group were: 1/5 @398 mg/kg, 2/5 @ 501 mg/kg, 4/5 @ 631 mg/kg, and 5/5 @ 794 mg/kg. Deaths occurred during study days 1-5, with most occurring during days 1-3. Clinical signs of toxicity observed included: increased weakness, slight tremors, ocular discharge. Necropsy of the viscera in decedents resulted in identification of lung hyperemia and discoloration of the liver, spleen and kidneys. Viscera of survivors (14 days) appeared normal.

Test substance :

p-Chloronitrobenzene (CASNO 100-00-5)

Reliability : (2) valid with restrictions

Study conducted prior to codification of OECD guideline 401 or inception of US GLPs (1979). Fewer animals used than stipulated in 401. Test results are highly consistent with 17 other rat OLD50 values ranging betwen 294-830 mg/kg as found in the ECB IUCLID PNCB, 2000.

Flag : Critical study for SIDS endpoint

31.05.2004 (12)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	inhalation
Exposure period	:	6 hr/day
Frequency of treatm.	:	5 days per week for 13 weeks
Post exposure period	:	
Doses	:	0, 1.5, 3, 6, 12 and 24 ppm
Control group	:	yes
NOAEL	:	< 1.5 ppm
LOAEL	:	= 1.5 ppm
Method	:	OECD Guide-line 413 "Subchronic Inhalation Toxicity: 90-day Study"
Year	:	1989
GLP	:	yes
Test substance	:	other TS

Method :

Groups of 10 male and 10 female F-344 rats were exposed in whole body stainless steel and glass chambers to vapors containing 0, 1.5, 3, 6, 12 or 24 ppm PNCB for 6 hr/d, 5 days per week, for 13 weeks. Vapor was generated by transfer of bulk PNCB into a flask and attached to a vapor generator with a rotary evaporation system. The resulting vapor was forced into a condenser and temperature maintained by circulating oil. Generator output and flow were automatically controlled. Chamber monitoring was performed using a GC/EC system. Low volatility of PNCB limited the maximum exposure vapor concentrations to the top level used in this study. Animals were individually caged, food and water administered ad libitum, and a 12 hr light:dark cycle employed. All animals were assessed for morbidity and mortality daily and weekly examined for clinical toxicity and recording of body weights. At termination of the study (13 weeks) all animals were necropsied and a full set of over 40 tissues and organs were examined microscopically for all high dose and control animals; target organs were examined for animals from lower dose groups. Organ weights and relative weights were assessed for all animals after 13 weeks of testing and included the following organs: heart, kidney, lung, liver, spleen, testis and thymus. The following hematology parameters were assessed on study day 1 (Methemoglobin only), 4, 23, and at 13 weeks from all rats from each study group: HCT, HGB, RBC, RETIC, MCV, MCH, MCHC, PLAT, WBC, MET, and WBC differentials. Similary, the following clinical chemistry parameters were measured from all rats at similar time points as hematology: BUN, CREAT, TPROT, ALB, GLOB, ALT, AP, CK, SDH, and bile acids. Williams parametric multiple comparison procedure was employed to statistically assess group-wise comparison of organ and body weights. Shirley's test for nonparametric analysis was used for clinical chemistry and hematology assessments. $P<0.05$ and <0.01 were used in all cases.

5. Toxicity

Id 100-00-5
Date 01.06.2004

Remark :

Sperm morphology and vaginal cytology evaluations were performed on rats exposed to 0, 6, 12, or 24 ppm PNCB. Male rats exposed to 24 ppm exhibited significantly lower left epididymal, cauda epididymal, and testis weights and lower spermatid heads/testis, spermatid counts and spermatozoal concentrations than control rats; estrous cycle length was decreased in all groups of PNCB-exposed females.

Result :

Mean concentrations in all test chambers were between 99-100% of target concentrations. No treatment related deaths, obvious clinical signs of toxicity, or effects on body weight were observed at any dose level. Hematology findings were consistent with methemoglobinemia and macrocytic (increased MCV) and hyperchromic (MCHC increase) hemolytic anemia seen at all test levels. Compensatory hematopoietic cell proliferation was present and considerable hemosiderin deposition observed microscopically, and produced a pattern of effects observed with other MET-forming agents. Following are the various statistically elevated/depressed effects noted at each dose level: At 1.5 ppm = increased MET, normocytic RBC (F only) and decreases in HCT, HGB, RBC, ALT (M only), renal hyaline droplet formation (males only), splenic congestion and hemosiderosis; at 3 ppm = increased MET, RETIC, normocytic RBC and bile acids (M only), and decreases in HCT, HGB, RBC, ALT, (M only), AP (F only), marked increase in spleen wt and mild liver wt (F only), renal hyaline droplets (M only), bone marrow hematopoietic cell proliferaton, Hardarian gland inflammation, congestion and hemosiderosis of the spleen along with hematopoietic cell proliferation and capsular fibrosis and hemosiderosis of the liver Kupfer cells (F only); at 6 ppm = increases in RETIC, MET, n-RBC, bile acids (M only), SDH (F only), MVC, spleen and liver weights and decreases in HCT,RBC, ALT, AP, TPROT, GLOB, and renal hyaline droplet formation (M only), bone marrow and splenic hematopoietic cell proliferation, Hardarian gland inflammation, splenic congestion, hemosiderosis and capsular fibrosis of the liver; at 12 ppm = decreases in HCT, HGB, RBC,AP, GLOB, ALT, TPROT and increases in MET, RETIC, n-RBC, SDH and bile acids, marked increases in spleen weights and mild increases in liver, heart and thymus weights, renal hyaline droplet formation (M only), bone marrow and splenic hematopoietic cell proliferation, Hardarian gland cell proliferaton, splenic congestion, hemosiderosis, and capsular fibrosis and hemosiderosis and histiocytic hyperplasia of the liver; at 24 ppm = increases in MET, RETIC, MCV, n-RBC, HGB, SDH, bile acids and decreases in HCT, HGB, RBC, AP, GLOB, ALT, TPROT, organ weight increases of the spleen, liver, heart, thymus and decreased testes weight, renal hyaline droplet formation (M only), bone marrow and splenic hematopoietic cell proliferation, Hardarian cell proliferation, splenic congestion and capsular fibrosis, hemosiderosis of the spleen and liver, histiocytic liver hyperplasia and testicular atrophy

5. Toxicity

Id 100-00-5
Date 01.06.2004

Test substance	:	PNCB determined to be > 97 % pure.
Reliability	:	(1) valid without restriction Well documented study consistent with OECD test guideline 413
Flag	:	Critical study for SIDS endpoint

31.05.2004 (13)

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	6 hr/day
Frequency of treatm.	:	5 days/week for 4 weeks
Post exposure period	:	
Doses	:	0, 5, 15, and 45 mg/m3 (equivalent to 0.78, 2.3 and 7 ppm)
Control group	:	yes
NOAEL	:	< 5 mg/m³
LOAEL	:	= 5 mg/m³
Method	:	OECD Guide-line 412 "Repeated Dose Inhalation Toxicity: 28-day or 14-day Study"
Year	:	1982
GLP	:	yes
Test substance	:	other TS

Method :

Groups of 10 male and 10 female SD rats were exposed via whole body in stainless steel and glass inhalation chambers to airborne concentrations of 0, 5, 15 or 45 mg/m3 PNCB for 6 hr/day, 5 days/week for 4 weeks. PNCB was mixed with a solvent and fed into a spray atomizer through which dry air was passed. Test material flow into test chambers was controlled using a fluid metering pump. Concentrations of PNCB were determined at least 3X daily using UV spectrophotometer; particle size distribution was determined throughout the study. Parameters monitored in this study included daily morbidity and mortality checks, weekly detailed clinical observations, and body weights. Hematology parameters (HGB, RBC, HCT, RETIC, MET, clotting time, RBC morph. and total and differential leukocytes) and clinical chemistries (BUN, SGPT, AP, GLU, ALB, TPROT, GLOB, K, CL, CA, PHOS) were analyzed at day 0 and just prior to termination (MET also analyzed after 2 weeks of testing) for 10 rats/sex/group. Ophthalmoscopic exams were conducted on all rats prior to study start and at termination. Organ (brain, testes, ovaries, heart, kidneys, pituitary, liver, lungs, spleen) weights and weight ratios were recorded at terminal sacrifice for all rats on test. Microscopic examination of over 40 tissues and organs were performed on all rats from the high dose and control groups at the end of the study. Spleens of all low and mid dose animals were also examined microscopically. Gormori's stain was used to semiquantitate the degree of hemosiderosis. A Bartletts' test was performed on study data to determine the degree of equality of variances (Snedecor and Cochran) followed by

Dunnet's test for parametric parameters and the Kruskal-Wallis test along with Dunn's Summed Rank test for nonparametric parameter analysis. P <0.05 was used in all cases.

Result :

Cumulative mean analytical exposure concentrations were 5, 16 and 45 mg/m3. Particle size distribution of the generated atmospheres established that PNCB was introduced as a vapor, rather than as an aerosol. No mortalities were observed in treated groups and mean body weights of PNCB-treated animals were similar to control values. Clinical signs of toxicity observed included: cyanosis of the conjuctivae, nasal areas and entire body in all three groups, with incidence increasing with dose. Other than a dark red appearance, no ocular abnormalities related to treatment were observed. Rats at all test levels exhibited slight reductions in HGB, HCT, RBC at one or both study intervals. Animals in the mid and high dose groups also exhibited an increase in the incidence of poikilocytosis and polychromia at the interim bleeding. MET showed a dose-related increase with levels approximating 2-8X controls. An increase in leukocytes was attributed to the abberant inclusion of reticulocytes in the automatic counting procedure for white blood cells. Small increases in GLU and reduced PHOS levels were seen in HD females only. Statistically elevated spleen and liver weights were seen in HD males and females (and rel. liver wts in mid dose females). An increased incidence of congestion, extramedullary hematopoiesis and hemosiderosis of the spleen was observed in male and female rats exposed to 45 mg/m3 and iron-positive pigmentation (hemosiderosis) in spleens of rats from the mid and low dose.

Test substance :

p-Chlorobenzene, greater than > 99 % pure

Reliability : (1) valid without restriction

Provided as Supplemental information as a longer-term study, conducted by the same exposure route, has been selected as the Key study for this HPV Endpoint; this study meets OECD Test Guidance 412 and was conducted under GLPs.

Flag : Critical study for SIDS endpoint

31.05.2004 (14)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	Salmonella typhimurium strains TA100, TA98, TA1535, TA1537
Test concentration	:	10, 4, 3, 1.5, 1.3, 1, 0.3, 0.2, 0.04, and 0.01 mg/plate
Cycotoxic concentr.	:	3 mg/plate
Metabolic activation	:	with and without
Result	:	positive
Method	:	OECD Guide-line 471
Year	:	
GLP	:	yes
Test substance	:	

Method :

Method used was plate incorporation assay based on Ames test methods consistent with OECD 471. All tests were run in duplicate and three plates were assayed at each dosage for each run both with and without metabolic activation. The S-9 liver homogenates were prepared from male SD rats and CD-1 mice given Arochlor 1254. All tester strains were obtained from Dr. B. Ames. Sterile DMSO was used as the solvent and a solvent control was employed of 20 uL/plate DMSO. Positive controls used were: 2-aminoanthracene, 9-aminoacridine, benzo(a)pyrene, NaNo2 and 2-nitrofluorene. A positive response was determined upon observation of a statistically significant dose-response increase in revertant colonies. Bartlett's test was used for pairwise comparison to controls and dose response determined using regression analysis for log-log straight lines; P<0.01 was used.

Result :

A definitive positive response was observed with TA1535 without metabolic activation, with some indication that TA1535 with metabolic activation was marginally positive.

Test substance :

p-Chloronitrobenzene, CASNO 100-00-5, purity greater than 99%

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
25.04.2004 (15)

Type	:	Cytogenetic assay
System of testing	:	Chinese Hamster Ovary Cell in vitro assay
Test concentration	:	50 to 5000 ug/mL
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: NTP
Year	:	
GLP	:	yes
Test substance	:	

Method :

Study conducted according to NTP study design, testing involved 3 separate tests (2 with S9 and 3 without S9 fraction added) SD male rat Arochlor 1254-induced liver homogenate was used. Cell cultures were handled to prevent

photolysis of Brdu-substituted DNA. Each test consisted of concurrent solvent and positive controls and at least 3 dose levels. Cells were incubated in McCoy's 5A medium with test agent ranging between 10.5-19 hrs, colcemid added and incubated for an additional 2 hrs and harvested/processed. 100 first-division metaphase cells were scored blind from prepared slides for each dose level. Classes of aberrations were recorded and included simple, complex and other abnormalities. Statistical analysis (Armitage trend test; Margolin multiple comparison test) were conducted on both the dose-response curve and individual dose points; significance was determined as $P<0.05$ for single data points and $P<0.015$ for trend.

Result :

Initial trials run at harvest times of 10.5 hr w and w/o S9 were negative. A follow up trial w/o S9 conducted at a higher dose level (700, 800 and 900 ug/ml) and incubated for 19 hrs (because PNCB induced cell cycle delay in the earlier study) resulted in an increase in aberrant cells only at 900 ug/plate; A repeat of this study at levels of 500, 600 and 700 ug/plate resulted in a dose related increase only at the top dose used. Repeat of the metabolic activation trial using a longer period to harvest (19 hr) produced an increase in aberrant cells.

Test substance :

4-Chloronitrobenzene, CASNO 100-00-5, from MC/B Chemical Co, Lot D11F12, practical grade, >99% pure by independent analysis.

Reliability : (2) valid with restrictions

Provided as Supplemental information as an in vivo cytogenetics study has been used to fulfill this HPV endpoint. Study considered reliable.

Flag : Critical study for SIDS endpoint

25.04.2004 (16)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Cytogenetic assay
Species	:	rat
Sex	:	
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	once
Doses	:	30, 100 and 300 mg/kg
Result	:	negative
Method	:	OECD Guide-line 475 "Genetic Toxicology: In vivo Mammalian Bone Marrow Cytogenetic Test - Chromosomal Analysis"
Year	:	1983
GLP	:	yes
Test substance	:	

Method :

Dose levels selected based on pilot study which produced 1/4 deaths @ 400 mg/kg and 4/4 deaths @ 600 mg/kg. Five rats/sex/time period were administered PNCB in corn oil by gavage. Metaphase cells were collected from rat bone marrow

(femur) at harvest times of 6, 12 and 24 hrs after treatment from 5 rats/sex. Colchicine was administered 2 hr prior to sacrifice to arrest cells in c-metaphase. Marrow was exposed to hypotonic solution and fixed, cells and slides prepared and stained. All slides were coded before reading. Positive (cyclophosphamide) and negative (corn oil) controls were used for comparative purposes. Mitotic index was calculated based on counting of at least 500 slides and all breaks, deletions, translocations and other changes recorded. Breaks or aberrations between treated vs control groups were compared by Chi-square analysis. P <0.05 was used.

Result :

Rats dosed with 100 and 300 mg/kg PNCB exhibited signs of cyanosis; animals given 300 mg/kg lost weight between time of dosing and sacrifice. No significant differences were observed in the frequency of breaks or aberrations between PNCB-treated and control groups at any of the three time points measured.

Test substance :

p-Chloronitrobenzene, CASNO 100-00-5, purity > 99%.

Reliability : (2) valid with restrictions

Time points measured did not include a period beyond 24 hr, but sufficient cells in metaphase were obtained at this time point that it was determined that there was no need to extend the sampling period.

Flag : Critical study for SIDS endpoint

01.06.2004 (17)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type : Two generation study
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : F0 & F1 Adults-premating through litter weaning (Fo) and postweaning (F1)
Frequency of treatm. : daily (7d/wk) gavage
Premating exposure period
Male : FO- 14 weeks; F1- 18 weeks
Female : FO-14 weeks; F1- 18 weeks
Duration of test : FO M/F - 167d; F1 M/F- 219d
No. of generation studies :
Doses : 0, 0.1, 0.7 and 5.0 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL parental : = .7 mg/kg bw
NOAEL F1 offspring : = 5 mg/kg bw
NOAEL F2 offspring : = 5 mg/kg bw
Method :
Year :
GLP : yes

Method :

Test material was administered to groups of 15M and 30F rats (vehicle control group also included) in corn oil to F0 and F1 generations during a premating (14 wks for F0 and 18 wks for F1) growth period, and through the ensuing mating, gestation and lactation intervals (1 litter/generation). F1 rats continued on treatment during a post-weaning period of 30d. Dosing concentrations analyzed by GC weekly for the first week of the study and monthly thereafter for accuracy Body weights were recorded weekly for F0 and F1M. For F0 and F1 F wts were recorded weekly through the growth period and up to mating, then resumed after mating until sacrifice. Food consumption was recorded weekly for F0 and F1 M from study start up to mating, then resumed after mating through study term. Food consumption for adult females F0 and F1 was recorded weekly through the growth period and again after weaning of litters. Cageside observations for morbidity and mortality were made weekly, as well as daily observations of clinical signs. Temperature, humidity and light-dark cycles were controlled. F0 adults were sacrificed following weaning of the F1 litters and given a gross postmortem examination; reproductive tissues (testes, epididymides, seminal vesicles) were evaluated histopathologically for all control and high dose males. Adult F1 M and F rats were sacrificed following completion of a post-weaning treatment interval, given a gross necropsy. A full histopathological examination of over 40 tissues and organs (including gonads) was performed on 10 randomly selected F1 adult animals/sex/group. Pups delivered to F0 and F1 females were evaluated for growth, survival and external irregularities during lactation days 0, 4, 14 and 21. F1 pups not selected for the adult generation were sacrificed and given a gross postmortem exam. Tissues were evaluated histopathologically (~40 tissues/organs) from 5/sex/group of F1 weanlings and F2 weanlings. Body weights and changes, food consumption, gestation length and number of offspring were analyzed using ANOVA techniques followed by Dunnet's Test for parametric parameters and Kruskal-Willis test followed by Dunn's Rank Sum for nonparametric analysis. Mortality and pregnancy rates, fetal and mating indices and pup survival were analyzed using Chi-square, followed by Fisher Exact test and Armitage's test for linear trend. The level of significance was reported at both the 5% and 1% levels.

Remark :

Slight decreases in male and female fertility indices, as well as testicular effects seen in 3 HD male rats in the FO generation are considered spurrious findings, unrelated to treatment. No such effects were noted in the F1 generation, which were exposed over a considerably longer dosing period. Likewise, no testicular effects were observed in a group of 50 male rats exposed to 5 mg/kg/d PNCB by gavage for 24 months, a dosing regimen similarly used here, albeit for substantively longer than the 14 weeks rats in the HD group in this study were dosed.

Result : Dosing solutions were confirmed analytically as accurately prepared. No treatment-related mortalities could be affirmed in this study, although several gavage-related deaths occurred sporadically. Mean body weights and weight gains of all FO male groups were similar; FO females exhibited slightly, but not statistically lower, body weights at all treatment levels. This finding was considered unrelated to treatment as there was no dose-response effect noted. Food consumption values were similar between treated and control FO males and females, except for HD females which consumed slightly, but not statistically signficantly, more food through the first 6 weeks of the study. The mating index (no. mating/total given opportunity to mate) were similar for all FO Males. The mating index for FO Females was 86.7, 80, 71.4 and 71.4%, respectively, from control through HD group; as all these values were within the historical control range for this indice in this laboratory, these findings were considered unrelated to treatment. No statistical differences were seen in either pregnancy rate or male fertility index between PNCB-treated and control animals from the FO generation. Three HD male rats in the FO parental generation were found to have testicular degeneration upon microscopic examination. FO dams treated with PNCB during gestation and lactation exhibited mean body weights and length of gestation indices comparable to control levels. The number of live and dead pups at birth and pup weights durng lacation of pups from FO dams were unaffected by PNCB dosing. Pup survival in the HD group was slightly, but statistically significantly lower than the control group. This finding was related to the complete loss of two litters in this group, a phenomenon experienced within the test lab on an infrequent, but not unusual, basis. Thus, this finding was judged unrelated to PNCB treatment. No compound-related gross postmortem changes were observed in the FO adults or F1 weanlings. F1 generation: No treatment-related effects were seen in any test group for survival, mean body weights and gains, and food consumption during mating, gestation and lactation. No treatment-related effects were observed during the gross postmortem evaluation of F1 adults. An increase in extramedullary hematopoiesis and brown pigmentation of reticuloendothelial cells of both sexes of the HD treated groups were observed following histological examination of F1 animals.All three PNCB-treated groups of F1 females exhibited a slightly (but not statistically) lower mating index than the control group; however, no dose-response was evident and the number of pregnant females in all groups, control and treated, were similar. Thus, this observation was not considered treatment-related. Male (FO1) mating and fertility indices were unaffected by treatment. Litter and pup survival indices in groups of F2 generation animals were comparable between all treated and control groups.Similarly, pup weights were unaffected by treatment at all test levels. No evidence of toxicity was observed during the gross postmortem evaluation of F2 pups and no compound-related changes were observed during histological examination of tissues in F1 and F2 weanling pups.

Test substance :

p-Nitrochlorobenzene, CASNO 100-00-5, Monsanto lot KM06 328, purity 99.43% (0.47% ortho isomer and 0.1% meta isomer)

Conclusion :

Systemic toxicity was observed in high-dose males and females evidenced as methemoglobinemia; thus, the NOAEL for systemic toxicity was considered to be 0.7 mg/kg-day

No adverse effects on reproductive endpoints were observed in any group and the reproductive NOAEL is considered to be 5 mg/kg-day.

Reliability : (1) valid without restriction

Well documented GLP study meeting OECD Test Guideline 416.

Flag : Critical study for SIDS endpoint

31.05.2004 (18)

Type	:	other
Species	:	mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	gavage
Exposure period	:	105 days
Frequency of treatm.	:	daily, 7 days per week for 7 days prior to cohousing and 98 days of cohousing
Premating exposure period		
Male	:	7 days
Female	:	7 days
Duration of test	:	98 days of continuous breeding
No. of generation studies	:	
Doses	:	0, 62.5, 125 and 250 mg/kg/d
Control group	:	yes, concurrent vehicle
Method	:	other
Year	:	
GLP	:	yes
Test substance	:	

Method :

Standard Continuous breeding protocol designed by NTP and published as Lamb, 1985. J. Amer. Coll. Toxicol. 4:163-171. Based on 2-week toxicity test to establish dose levels, animals are individually housed for 7 days, then cohoused in breeding pairs for 98 days, and allowed to propagate. During this period the following indices are recorded: clinical signs of toxicity, mortality, parental body weight and average consumption of water during representative weeks, fertility (e.g. no. of pairs producing a litter/number of breeding pairs), the no. of litters per pair, the no. live pups/litter, % pups born alive, sex ration of pups and pup body weights after birth). The last litter born during the holding period (5 weeks) following the breeding period is reared until weaning after which treatment of the F1 animals was initiated and these animals used for assessment of second generation fertility. For this phase, siblings were cohoused until sexual maturity, when 20 non-sibling males and females per treatment group were cohabitated for 7 days and then housed singly through delivery. Endpoints for this

mating trial were the same as for the F0 generation. At termination of F0 and F1 generations, animals were necropsied and evaluations made for organ weights (ovaries or testes and epididymides from 5 per group, control and HD), body weights, epididymal sperm motility, sperm morphology, sperm count and estrual cyclicity. Methemoglobin measurements and spleen weights were recorded for both the F0 and F1 generations. Proportional data were assessed statistically using the Armitage trend test, with each dose group compared to control using a chi-square analysis. Absolute body and organ weights were compared using Shirley's or Dunn's test while dose related trends were identified by Jonckheere's test. Vaginal cytology was analyzed using an analysis of variance described by Morrison to test for simultaneous equality. A p value of <0.05 or <0.01 was used.

The following reproductive organs were weighed and examined microscopically as appropriate.
- Ovaries
-Testes
-Epididymis
-Seminal vesicles
-Prostate

Result :

Fertility of mice dosed with PNCB decreased progressively with the duration of dosing and with increasing dose and being statistically different from controls at the high dose level. Most mice exposed to 250 mg/kg were cyanotic. Spleen and liver weights of F1 PNCB-treated mice reportedly were significantly greater than those of the controls. Survival and body weights of F1 (final litter) and F2 pups were significantly decreased at 250 mg/kg and at 125 mg/kg (F1 only).

NOAEL's are difficult to specify in this study and may be less than 62.5 mg/kg-day for doth systemic effects and repeoductive effects.

Test substance :

p-Nitrochlorobenzene, CASNO 100-00-5, purity 97%

Reliability : (1) valid without restriction

GLP study, peer reviewed and followed an established study design.

Flag : Critical study for SIDS endpoint

27.04.2004 (13)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	once per day
Frequency of treatm.	:	gestation days 6 through19
Duration of test	:	rats sacrificed on gestation day 20
Doses	:	5, 15 and 45 mg/kg/day

Control group	:	yes
NOAEL maternal tox.	:	< 5 mg/kg bw
NOAEL teratogen.	:	= 15 mg/kg bw
NOAEL Fetotoxicity	:	= 15 mg/kg bw
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	1980
GLP	:	yes
Test substance	:	other TS

Method :

Groups of 24 mated female rats were dosed daily by gavage (test material dissolved/suspended in corn oil) during gestation days 6-19. All rats were observed for mortality and abnormal behavior twice daily from gestation day 0 through day 20, at which time all animals were sacrificed and maternal spleen weights recorded. Detailed physical exams for signs of toxicity were recorded on study days 0, 6, 10, 15 and 20. Maternal body weights were recorded at several intervals throughout the study. At sacrifice the uterine horns were examined for implantation sites, resoptions and the number of viable or non-viable fetuses. The number of corpora lutea were also recorded. The sex and weights of all live fetuses were recorded and all fetuses were examined for external abnormalities. One-half of the fetuses per litter were examined for skeletal malformations while the other half were examined for internal anomalies. The following statistical analyses were performed: For interval data (body wts, wt changes, reproductive data) Bartlett's test was used to determine equality of variance and ANOVA and Dunnett's test used for parametric data while the Kruskal-Wallis test and Summed Rank test used for nonparametric data (Snedecor and Cochran). For Incidence data, i.e. mortality rates, % and incidence of variations and malformations comparisons were made using the Chi-square contingency table and the 2X2 Fisher Exact test employing the Bonferroni inequality estimate; linear trend was evaluated using the Armitage test. Comparisons were made using the litter as the comparative entity. Both the 5% and 10% level of statistical significance were reported for each parameter.

Result :

Maternal toxicity (reduced body weight gain during the treatment period and increased spleen weights), fetotoxicity (increased no. resorptions/litter), embryotoxicity (increased no. fetuses with unossified sternebrae, incompletely ossified cervical and vertebral transverse processes) and fetal skeletal (predominantly angulated ribs) malformations were observed at the 45 mg/kg dosage level. At 15 mg/kg, similar maternal toxicity but no fetotoxic/embryotoxic or teratogenic responses were observed. At 5 mg/kg, only a slight increase in spleen weight was observed in maternal animals.

Test substance :

p-Nitrochlorobenzene, CASNO 100-00-5, Monsanto lot KM06 328, purity 99.43% (0.47% ortho isomer and 0.1% meta isomer)

Conclusion :

PNCB produced teratogenic effects only at dosages which

produced significant maternal toxicity.

Reliability : (1) valid without restriction

This study meets OECD Test Guideline 414 and was conducted under GLPs.

Flag : Critical study for SIDS endpoint

27.04.2004 (19)

Species	:	rabbit
Sex	:	female
Strain	:	New Zealand white
Route of admin.	:	gavage
Exposure period	:	gestation days 7 through 19
Frequency of treatm.	:	once daily
Duration of test	:	animals sacrificed on gestation day 30
Doses	:	0, 5, 15, 40 mg/kg
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 15
NOAEL teratogen.	:	= 15
Method	:	OECD Guide-line 414 "Teratogenicity"
Year	:	
GLP	:	yes
Test substance	:	

Method :

Groups of 18 mated female NZ white rabbits were administered PNCB in corn oil (constant volume of 2 ml/kg) in corn oil at PNCB concentrations of 0 (vehicle control), 5, 15, and 40 mg/kg on gestation days 7-19. Animals were evaluated for detailed signs of toxicity on test days 0, 7, 10, 15, 19 and 30; body weights were recorded on test days 0, 7, 19 and 30. Daily observations were made for morbidity and mortality. Food and water were administered ad libitum and a 12 light:dark cycle was employed. Temperature and humidity were controled. All animals were examined externally and 1/2 were evaluated for soft tissue malformations and the other 1/2 for skeletal findings. The following statistical analyses were performed: For interval data (body wts, wt changes, reproductive data) Bartlett's test was used to determine equality of variance and ANOVA and Dunnett's test used for parametric data while the Kruskal-Wallis test and Summed Rank test used for nonparametric data (Snedecor and Cochran). For Incidence data, i.e. mortality rates, % and incidence of variations and malformations comparisons were made using the Chi-square contingency table and the 2X2 Fisher Exact test employing the Bonferroni inequality estimate; linear trend was evaluated using the Armitage test. Comparisons were made using the litter as the comparative entity. Both the 5% and 10% level of statistical significance were reported for each parameter.

Result : Mortality so high at 40 mg/kg level that this study group was terminated without additional data collection. 15 mg/kg and 5 mg/kg- no effects on survival or maternal body wts, no treatment-related effects in uterine implantation data, fetal wts or sexing data. No statistically significant differences seen in skeletal malformations between treated and control groups nor was there any treatment-related

increase in the incidence of external or soft tissue findings.

Test substance :

p-Nitrochlorobenzene, CASNO 100-00-5, Monsanto lot KM06 328, purity 99.43% (0.47% ortho isomer and 0.1% meta isomer)

Reliability : (1) valid without restriction

Well conducted study following GLP guidance and OECD study design. Limited due to excessive no. of deaths at the high dose group which disallowed any developmental toxicity information to be obtained from this study group.

Flag : Critical study for SIDS endpoint

27.04.2004 (20)

9. References

(1) Lide, DR (ed). CRC Handbook of Chemistry and Physics. 1990-1991. 71st edition. CRC Press Inc. Boca Raton, FL.

(2) Bessarab, NA, FS Chemoglazova and YM Martynov. 1973. Vapor pressure of some chloro derivatives of nitrobenzene. Zh. Fiz. Khim. 47:1048.

(3) Nimi, AJ, HB Lee and GP Kissoon. IN Devillers, J, S Bintein and D Domine. 1996. Comparison of BCF models based on log P. Chemosphere 33(6):1047-1065

(4) Kuehne, R, R-U Ebert, F Kleint, G Schmidt and G Schuurmann. 1995. Group contribution methods to estimate water solubility of organic chemicals. Chemosphere 30(11):2061-2077.

(5) Kanno, S and K Nojima. 1979. Studies on photochemistry of aromatic hydrocarbons. V. Photochemical reaction of chlorobenzene in air. Chemosphere 4:225-232.

(6) EPIWIN. 2002. Version 3.1. Syracuse Research Corp., Syracuse, NY.

(7) Michael Smith and Jerry March. March's Advanced Organic Chemistry, Wiley-Interscience, fifth ed 2001 page 433

(8) Solutia study no. 3819. Final Report on Analytical chemistry Investigations - 1971 Special Studies.

(9) Solutia study no. AB-80-315. Acute Toxicity of p-Nitrochlorobenzene to Rainbow Trout.

(10) Solutia study no. MO-83-X078. Acute Toxicity of Para-nitrochlorobenzene to Daphnia magna.

(11) Kuhn, R and M Pattard. 1990. Results of the harmful effects of water pollutants to green algae (S. subspicatus) in the cell multiplication inhibition test. Water Res. 24(1):31-38.

(12) Solutia study no. Y-75-48, Toxicological Investigation of p-Nitrochlorobenzene.

(13) NTP. 1993. Toxicity Report Series, Number 33 - NTP Technical Report on toxicity studies of 2-Chloronitrobenzene and 4-Chloronitrobenzene. NIH Publication 93-3382.

(14) Solutia study no. BD-81-106. A Four Week Inhalation Toxicity Study of p-Nitrochlorobenzene in the Rat; [EPA Document no. FYI-OTS-1085-0455}[also found in Nair el al. 1986. Fundamen Appl Toxicol 6:618-627].

(15) Solutia study no. DA-79-258. Salmonella mutagenicity assay of CP 6560 [EPA Document no. 86940000672].

(16) NTP, 1993. Toxicity Report Series, Number 33-NTP Technical Report on toxicity studies of 2-Chloronitrobenzene and 4-Chloronitrobenzene. NIH Publication 93-3382 [also Galloway, et al. 1987. Environ Mol Mutagen 10:1-175].

(17) Solutia study no. BA-83-240. In vivo chromosomal aberration assay with p-Nitrochlorobenzene. [EPA Document No. 86940000673/TS-000055402].

(18) Solutia study no. BD-80-472. A Two Generation Reproduction Study of p-Nitrochlorobenzene in Rats. [EPA Document no. 86940000677/TS-00055406]

(19) Solutia study no. BD-79-327. A Teratology Study with p-Nitrochlorobenzene in Rats.[also found in Nair et al. 1985. in Toxicity of Nitroaromatic Compounds, Hemisphere Publ.].[EPA Document no. 86940000663/TS-00055402]

(20) Solutia study no. BD-80-530. A Teratogenicity Study in Rabbits with p-Nitrochlorobenzene. [EPA Document no. 86940000663].